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Neurokinin-2 receptor antagonism in medial septum influences temporal-order memory for objects and forebrain cholinergic activity

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ABSTRACT

In the mammalian brain the neurokinin NK_2 receptors are predominantly located in the hippocampus, thalamus, septum and frontal cortex. It has been shown that administration of the NK_2 receptor agonist, neurokinin A (NKA), into the medial septum of rats increases extracellular levels of acetylcholine (ACh) in the hippocampus and that NK_2 receptor antagonism blocks this increase. Therefore, given the prominent role of hippocampal ACh in information processing, we hypothesized that NK_2 receptor antagonism in the medial septum would negatively affect learning and memory via its influence on the cholinergic neurons of the basal forebrain. We investigated the action of local application of the peptidic NK_2 receptor antagonist, Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH (1, 10 and 100 pmol), into the medial septum on object memory for temporal order and spatial location using an object novelty paradigm. By means of in vivo microdialysis and HPLC analyses, we also examined the influence of NK_2 receptor antagonism in the medial septum on ACh in major cholinergic projection areas of the basal forebrain, namely, hippocampus, frontal cortex and amygdala. *Results*: Injection of vehicle alone into the medial septum impaired memory for temporal order and spatial location of objects. Application of 1 pmol of the NK_2 receptor antagonist partially reversed this deficit by reinstating memory for temporal order. Injection of 10 pmol of the NK_2 receptor antagonist into the medial septum decreased levels of ACh in the hippocampus (at 30 min post-injection), and frontal cortex (at 30 and 80 min post-injection) in comparison to vehicle. However, this apparent decrease was the result of the blockade of a saline-induced increase in ACh levels.

1. Introduction

Three neurokinin (NK) receptors have been identified in the brain: the neurokinin-1 (NK_1), NK_2 and NK_3 receptors. While NK_1 and NK_3 receptors are widespread in the brain, the NK_2 receptors are found in restricted brain areas, i.e., in the frontal cortex, hippocampus, shell of the nucleus accumbens, septum and thalamus. Neurokinin A (NKA), neuropeptide K (NPK) and neuropeptide γ (NPγ) are the preferred endogenous ligands for NK_2 receptors, although substance P and neurokinin B also bind with lower affinity [39,40].

Relatively little is known about the functions of NK_2 receptors. Based on receptor localization, NK_2 receptors are suggested to be involved in drug sensitization, addiction and schizophrenia [39]. NK_2 receptor antagonists have also been proposed for the treatment of depression and some forms of anxiety disorders [19,20,43].

The septo-hippocampal cholinergic system has been implicated in a number of behavioral processes, including arousal, memory and emotion [17,45,47]. It has also been proposed to function as a gating system in the processing and integration of signals derived from several sensory inputs [31]. NK agonists exert an excitatory influence on the septo-hippocampal cholinergic system [28]. Post-trial injection of SP into the medial septum was found to facilitate passive avoidance learning [42]. Administration of the NK_2 receptor agonist, neurokinin A (NKA), into the medial septum of rats increased extracellular levels of acetylcholine (ACh) in the hippocampus and NK_2 receptor antagonism blocked this increase [44]. A highly selective and potent NK_2 receptor antagonist significantly blocked the NKA-induced release of ACh [21]. The enhancing effects of NKA were mediated by the activation of NK_2 receptors, since they were antagonized by SR48968. These results indicate that the NK_2 receptor may be critically involved in regulating hippocampal ACh release. This finding, and the prominent role attributed to forebrain ACh in learning and memory processes [3,18,30], is suggestive of a role for the NK_2 receptors in learning and memory processes via cholinergic

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modulation. We, therefore, hypothesized that NK_2 receptor antagonism in the medial septum would negatively affect learning and memory by influencing the activity of the cholinergic neurons of its main projection area, the hippocampus. The effects of intra-septal NK_2 receptor antagonism on learning and memory performance have so far not been investigated.

In the present study, we first assessed the effects of pre-trial administration of a peptidergic NK_2 receptor antagonist on object recognition memory in a novelty-preference paradigm, which combines memory for temporal order [32] and memory for spatial location [15]. Based on the suppressive effects of NK_2 receptor antagonism on hippocampal ACh release induced by medial septal NK_2 receptor agonism [44], we expected that NK_2 receptor antagonism in the medial septum would disrupt performance on the object recognition tasks. Furthermore, we tested whether intra-septal NK_2 receptor antagonism would modulate extracellular ACh levels under basal non-stimulated conditions, as assessed by vivo microdialysis and high performance liquid chromatography with electrochemical detection (HPLC–ECD). Again, based on the findings of Steinberg et al. above [44], we expected this treatment to decrease levels of ACh, especially in the hippocampus, the main cholinergic projection from the medial septal area. ACh levels were also measured concurrently in other basal forebrain cholinergic projection areas, namely the frontal cortex and amygdala.

2. Methods

2.1. Subjects

Adult male Wistar rats (260–300 g at the beginning of the experiments) from the breeding colony of the University of Düsseldorf were used for all experiments. Animals were housed in translucent plastic cages (60.0 cm × 20.0 cm × 38.0 cm; length × depth × height) under controlled laboratory conditions (temperature: 20 ± 2 °C) with free access to food and water under an artificial reversed 12:12 light–dark cycle (light off at 07:00 am). They were housed in groups of five animals per cage until the implantation of the guide-cannulae was carried out. The animals were allowed to adjust to the housing conditions for 2 weeks and were handled daily for 5 days preceding the experiments. Experiments were performed during the animals' active period between 8:00 am and 5:00 pm. All experiments were carried out according to the German Law of Animal Protection of 1998.

2.2. Drugs

The peptidic NK_2 receptor antagonist used, Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (Bachem, USA), is extremely potent and selective [29]. It was diluted in phosphate-buffered saline (PBS) and administered in three doses: 1, 10, and 100 pmol. The vehicle, PBS, served as a control condition. For drug administration, an injection needle (17.1 mm long, 0.20 gauge) was inserted into the medial septum through a guide-cannula (16.3 mm long, 0.22 gauge) and the substance was applied in 0.5 μl volume with a flow rate of 1.0 μl/min, using a microinfusion pump (CMA/100, Sweden). After the end of the injection, the needle was left in place for another 30 s.

2.3. Object memory for temporal order and spatial location

We examined the effects of pre-trial administration of the NK_2 receptor antagonist, Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2, into the medial septal area on object recognition memory in a novelty-preference paradigm, which combines memory for temporal order and memory for spatial location of objects.

2.3.1. Surgery

For local application of drugs, 5–7 days prior to the beginning of the behavioral testing, the animals were anesthetized with a mixture of ketaminhydrochloride (90.0 mg/kg; Pharmacia & Upjohn GmbH, Germany) and xylazinhydrochloride (8.0 mg/kg; Bayer, Germany). With the help of a stereotaxic apparatus (David Kopf Instruments, USA) and according to a stereotaxic atlas [36], a stainless steel guide-cannula for microinjection of the antagonist (16.3 mm long, 0.22 mm gauge) was placed above the medial septum (AP: +0.82 cm; ML: ±0.16 cm; DV: −0.60 cm, with 15° angle toward the midline); coordinates were taken from bregma. The guide-cannulae were placed unilaterally, counterbalanced into the right or left hemispheres and were fixed with two stainless steel screws and dental cement. After surgery the animals were allowed to recover for 1 week before being subjected to behavioral testing. They were housed individually for 2 days and afterwards, two animals per cage.

2.3.2. Apparatus

Memory for temporal order of presentation and spatial location of objects was tested in an open-field arena. The open-field used was a rectangular box (60 cm × 60 cm × 30 cm) with grey acrylic walls and open roof, located in a sound-attenuating room with masking noise. The arena was illuminated by four 40 W bulbs that provided a light density of approximately 13 lx at the center of the field. Its floor was divided into virtual nine quadrants of equal size, which were the possible positions of object placement, except for the central square, which was not used for object placement. A video camera, connected to a video recorder, was mounted 1.6 m above the arena to record the experiment on video tapes for off-line data acquisition.

2.3.3. Objects

Two different objects (in quadruplicate), made of plastic material, differing in color (violet, colorless), shape (circular, rectangle) and surface texture (plain, grooved) were used. Since the objects were made of the same material, they could not be distinguished by olfactory cues emanating from the material with which the objects were made, during the test trial. The objects had a height of 28 cm and a sufficient weight (1500 g) to ensure that the rats could not displace them. Pilot studies ensured that rats could discriminate the two objects, and that there was no *a priori* preference for any of the objects.

2.3.4. Experimental procedure

This task combines different versions of the novelty-preference paradigm that are presumed to measure (a) object recognition memory [14], (b) memory for temporal order of presentation of objects [32] and (c) memory for locations in which objects were encountered [15].

Each animal was exposed to the open-field on 3 consecutive days (habituation). It was placed into the central part of the open-field and allowed to explore for 10 min. One day after the last habituation trial, the object memory test was administered. The animals received two sample trials, followed by the test trial (Fig. 1). The rats were always placed into the central part of the open-field, facing the same direction. Each trial lasted 5 min, with inter-trial intervals of 50 min. For each animal, four out of eight squares were randomly chosen to position the four copies of the "old" object in the first sample trial (see top of Fig. 1). During the second sample trial, four copies of a different object ("recent") were present. Two copies of the "recent" object were randomly placed onto positions that had been occupied in the first sample trial and two copies were placed in new positions, which were randomly chosen from the remaining six positions. In the test trial, two copies of both "old" (A1 and A2) and recent (B1 and B2) objects